Genetic Susceptibility of Chicken × Quail Hybrid Embryos to Avian RNA Tumour Viruses

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SUMMARY

An attempt was made to hybridize the chicken (Gallus domesticus) male with Japanese quail (Coturnix coturnix japonica) female in order to study the genetic susceptibility of hybrid embryos to avian RNA tumour viruses of subgroups, A, B, D and E. In the hybrids the results supported the prevailing concept that susceptibility is dominant over resistance regardless of the dominant trait contributed by either parent. It was also observed that the I\textsuperscript{e} gene of the chicken was unable to suppress the ‘quail-coded’ susceptibility to subgroup E virus in the hybrid system, suggesting the lack of penetrance of the I\textsuperscript{e} gene. Despite the fact that some hybrids were resistant to viruses of subgroups B and D, they were susceptible to subgroup E virus, which was not expected on the basis of the concept that subgroup B-resistant cells cannot be E-susceptible. Also, the hybrids were susceptible to E virus regardless of gs antigen expression and presence of the I\textsuperscript{e} gene in the genome. This indicates that our earlier suggestion that the I\textsuperscript{e} gene is another expression of the gs antigen-determining gene is inconsistent.

Chickens vary in their response to tumour viruses of subgroup A, B, D and E, depending on the dominant susceptibility or recessive resistance genes they carry (Payne, 1971). They are invariably resistant to E virus if they carry the inhibitor I\textsuperscript{e} gene (Payne, Pani & Weiss, 1971; Crittenden, Wendel & Motta, 1973; Pani & Payne, 1973; Pani, 1974). This I\textsuperscript{e} gene has the property of suppressing the expression of the e\textsuperscript{s} susceptibility gene, making the e\textsuperscript{s}-carrying individual phenotypically resistant. Group specific (gs) antigen expression in the chicken is under the control of a pair of autosomal alleles, gs\textsuperscript{+} and gs\textsuperscript{−}, with gs\textsuperscript{+} being dominant over the gs\textsuperscript{−} (Payne & Chubb, 1968). The two genes I\textsuperscript{e} and gs\textsuperscript{+} are believed to be related to the expression of endogenous subgroup E virus (Weiss et al. 1971; Hanafusa et al. 1972; Pani & Payne, 1973). Although it was postulated earlier by us (Payne et al. 1971) that the I\textsuperscript{e} gene is either linked with, or another expression of, the gs\textsuperscript{+} gene, this study presents evidence against this suggestion.

Japanese quail (Coturnix coturnix japonica) are susceptible to subgroup E virus, segregate in response to subgroup A virus and are resistant to subgroup B, C and D viruses. In addition they do not express gs antigen (Tooze, 1973; P. K. Pani, unpublished data). Nothing is known of the way in which these traits are inherited, and therefore it is not possible at present to identify tumour virus loci in quail comparable to those in the chicken.

Chicken (Gallus domesticus) and Japanese quail (Coturnix coturnix japonica) belong to the same subfamily (Phasianinæ) but to different genera, and hybridization between the two species has been successful but with low fertility (7.4%) and hatchability (0.4%) rates (Wilcox & Clark, 1961).

In view of the differences in response of chickens and Japanese quail to avian RNA tumour viruses (ARTV), it was of interest to investigate (a) the response of hybrids between these species to these viruses, and (b) which of the two genetic systems, chicken or quail, controls
the response of the hybrids and particularly what role the \( I^e \) and \( gs^+ \) genes play in the hybrid system.

Hybridization was performed by artificial insemination of 6- to 7-week-old female quail with chicken semen. Reciprocal hybridization was not attempted because of difficulties encountered in the collection and handling of semen from the male quail. To achieve this, 20 to 25 female quail, separated from contemporary males at 25 to 28 days of age, were allocated to each of three chicken male lines. A pen of the same number of female quail with 10 male quail was kept to provide control embryos. The quail flock was raised from three pairs of breeding parents and at the present time is in the 34th generation of random mating. Male chickens were from the Reaseheath lines and their crosses maintained at Houghton Poultry Research Station.

The procedure of semen collection, insemination of the female quail, and handling of eggs during incubation was as described by Wilcox & Clark (1961). Semen from 4 to 6 cocks of each chicken male line was collected and pooled and each female quail was inseminated once a week with 0.05 ml. Three male lines of known tumour virus genotypes (Payne & Biggs, 1966; Payne & Pani, 1971; Pani, 1974) and gs status (Payne & Chubb, 1968; Weiss & Payne, 1971) were used as follows:

1. \( I \times W (F_2) : a'^a'b'^b'I^e'e^e gs^+g^- \)
2. \( W \times C (F_1) : a'^a'b'^b'I^e'e^e g^-g^- \)
3. Reaseheath C line: \( a'^a'b'^b'i'e^e e^- e^- g^- g^- \)

The \( IW(F_2) \) male line is homozygous for the dominant genes, \( a'^a' \), \( I^e \) and \( gs^+ \) at the \( tva \) (tumour virus a), \( inhibitor^+ (I^e) \) and gs antigen determining loci respectively, but is segregating for the \( b'^b' \) genes at the \( tvb \) locus and for the \( e'^e' \) genes at the \( tve \) locus. The C male line is homozygous for the recessive genes at the loci under consideration whereas the WC male line is heterozygous for the genes at each locus except the \( tve \) where it is homozygous recessive for the \( e'^e' \) gene.

Hybrid embryos were cultured and challenged in accordance with the procedure adopted for chicken embryos (Temin & Rubin, 1958). Ten-day-old hybrid embryos and 8-day-old quail embryos were cultured individually. Duplicate secondary cultures were challenged with 0.2 ml of BS-RSV (subgroup A), RSV(RAV-2) (subgroup B), RSV(RAV-50) (subgroup D) (Payne & Biggs, 1966; Pani, 1975) and RSV(RAV-0) (subgroup E) (Payne et al. 1971; Pani & Payne 1973), in appropriate dilutions in phosphate buffered saline. The inoculum contained 4000 focus forming units (f.f.u.)/ml of viruses of subgroup A, B and D and, 3000 f.f.u./ml of subgroup E. For infection of cultures with subgroup E virus the secondary cells were cultured in medium containing polybrene (2 \( \mu g/ml \)) and without calf serum. After 4 to 6 h the cells were challenged with RSV(RAV-0) for 1 h, re-fed with secondary medium containing 5% calf serum, and after 10 to 12 h overlayed with nutrient agar containing inactivated 1% chick serum and 1% dimethylsulphoxide. Foci were counted 10 days after infection. Brown Leghorn chicken cells susceptible to viruses of A, B and D subgroups and quail cells susceptible to E subgroup were used to check on the doses of viruses used.

Prior to the culture of cells of hybrid and quail embryos the viscera from each embryo were collected for the complement fixation of avian leukemia (COFAL) test (Payne & Chubb 1968; Sarma, Turner & Huebner, 1964) to determine the gs status of each embryo.

The average focus counts of individual hybrid embryo cultures challenged with ARTV of subgroups A, B, D and E and the gs status are shown in Table 1. The striking finding of the study was the susceptibility of the hybrids to subgroup E. All the hybrids, regardless of chicken male line, were susceptible to subgroup E. Because the
Table 1. Focus counts on cultures of individual chicken × quail hybrid embryos and of quail embryos challenged with ARTV of subgroups A, B, D and E and phenotypic segregation patterns of resistance (R) and susceptibility (S) and of gs-antigen (gs-a) expression within each male line

<table>
<thead>
<tr>
<th>Type of cross</th>
<th>Male line</th>
<th>Embryo no.</th>
<th>Response to virus subgroup</th>
<th>Presence (+) or absence (−) of gs antigen</th>
<th>Phenotypic segregation pattern*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken × quail</td>
<td>IW(F2)</td>
<td>1</td>
<td>140 TNC† TNC 163</td>
<td>+</td>
<td>S/R S/R S  gs-a+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>TNC 0 5 TNC</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>TNC 5 3  TNC</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>TNC 4 14 TNC</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>153 TNC TNC TNC</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>175 TNC TNC 95</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>TNC TNC TNC −</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>TNC TNC TNC −</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>TNC 1 R</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>TNC TNC 160</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Chicken × quail</td>
<td>WC(F1)</td>
<td>1</td>
<td>150 2 9 82</td>
<td>+</td>
<td>S R R S  gs-a+</td>
</tr>
<tr>
<td>Chicken × quail</td>
<td>C line</td>
<td>1</td>
<td>TNC TNC TNC 95</td>
<td>−</td>
<td>S S S S  gs-a−</td>
</tr>
<tr>
<td>Quail × quail (control)§</td>
<td>Quail</td>
<td>1-22</td>
<td>O-TNC 0 0 74-TNC</td>
<td>−</td>
<td>S/R R R S  gs-a−</td>
</tr>
</tbody>
</table>

* The observed phenotypic segregation patterns agreed well with the expected segregation patterns except for the response to subgroup E virus.
† TNC = too numerous to count (at least 350 foci).
†† Cell sheet died.
§ Twenty two quail control embryos with range of focus counts.

IW male line was of I+I+ genotype, hybrids from this line were not expected to be susceptible to subgroup E virus because the I+ gene should have suppressed the phenotypic expression of the genetically susceptible hybrid embryos. This indicates that the I+ gene is not penetrant in chicken × quail hybrid system or that 'quail-coded’ susceptibility is dominant over ‘chicken-coded’ susceptibility to subgroup E.

It was also observed that hybrid embryos having low focus counts in response to subgroup B virus also had low counts to subgroup D virus (embryos no. 2, 3, 4 and 9 of the IW(F2) I line and embryo no. 1 of the WC(F1) I line). These low counts (fewer than 15 foci) can be regarded as the phenotypic expression of the resistant hybrid to B and D subgroups. Despite the low counts to B and D subgroups they were highly susceptible to subgroup E. This evidence indicated that B-resistant hybrid cells can be infected with subgroup E virus, as can pure quail cells (Table 1). This contradicts the hypothesis advanced by Crittenden et al. (1973) that B and E subgroup resistances are associated. Nevertheless, this type of association could be restricted to chickens only.

Since the IW(F2) and WC(F1) male line chickens are b+ and b+ b+ genotypes respectively the segregation of resistant and susceptible phenotypes was observed as expected. Also it is concluded that the susceptibility alleles of the chicken for B and D subgroup viruses are dominant over the resistance alleles of the quail.

Chicken × quail hybrid cells resistant to subgroup B virus were also resistant to subgroup D virus (Table 1), suggesting that B and D responses are probably controlled by the same set of genes. This is in accord with evidence recently reported for the chicken (Pani, 1975).

Quail cells varied in response to subgroup A. Out of 22 control embryos (Table 1), more
than 50% had focus counts of 10 or less. Very few had high counts (‘TNC’). On the other hand, most of the hybrids were highly susceptible. Also, despite the \(a'a''\) genotype of the C male line chickens, the hybrids within the line were highly susceptible. Since all the female quail originated from the same stock it is difficult to explain the higher proportion of susceptible hybrids. Possibly the genes controlling subgroup A response in chickens are not at the same locus as those controlling the same response in quail. If so, complementary gene action could result in hybrid superiority over the parents with respect to susceptibility to subgroup A virus. However, considering the sample size more work is needed to resolve this question.

The gs status of the individual hybrid embryos clearly indicated that it is determined by a gene which is common to chicken and quail. The observed results were in agreement with the expectation based on knowledge of the behaviour of gs genes in chickens. The IW and C male line chickens are of \(gs^+gs^+\) and \(gs^-gs^-\) genotypes respectively. Since all the hybrids of the IW male line were COFAL positive and those of the C male line COFAL negative, it is concluded with caution, due to very few embryos of the C male line, that the chicken \(gs^+\) gene is dominant in the hybrid.

On the basis of the results the patterns of segregation of resistance and susceptibility to ARTV and of gs antigen expression within each male line are also presented in Table 1. In this study the most important single conclusion is consistent with the prevailing concept in chickens, and is that susceptibility is dominant over resistance, whichever parent contributes the dominant trait. Curiously, in the hybrids the dominant \(I^e\) gene of the chicken system could not suppress the susceptibility to subgroup E virus.

In chickens two possible mechanisms of inhibition of the phenotypic expression of the \(e^\) gene by the \(I^e\) gene have been suggested: (1) a product of the \(I^e\) gene may block the \(e^\)-coded virus receptor on the cell surface (Payne et al. 1971); (2) a virus moiety under the control of the regulatory \(I^e\) gene may act as a repressor substance on the operator of the operon which codes for the subgroup E virus receptor (Pani & Payne, 1973). However, neither of these mechanisms appears to operate in the chicken–quail hybrids which were susceptible to subgroup E virus even though carrying the \(I^e\) gene. There are at least two possible reasons for this. (1) There may be lack of penetrance of the \(I^e\) gene in the hybrid, possibly because the genetic environment differs from that of the parents. This may be comparable to the lack of expression of chicken comb genes in the hybrid (Wilcox & Clark, 1961; P. K. Pani & P. C. Powell, unpublished data). (2) ‘Quail-coded’ susceptibility to subgroup E virus may be dominant over ‘chicken coded’ susceptibility in the hybrid, and the epistatic action of the \(I^e\) gene may be restricted to chicken genes. One additional point worth mentioning is that regardless of gs antigen status, the chicken–quail hybrids were susceptible to subgroup E virus. This supports the evidence of Crittenden et al. (1973) that there is no association between gs antigen status and susceptibility to subgroup E, contrary to the suggestion originally made by Payne et al. (1971).

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